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ENVIRONMENTAL RESEARCH

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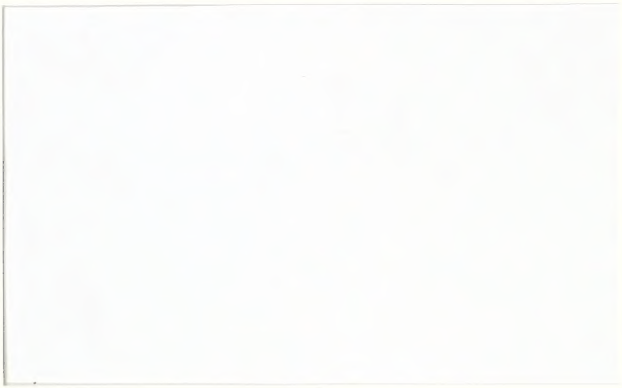
ACUTE AND CHRONIC TOXICITY TO ZOOPLANKTON OF PURE PENTACHLOROPHENOL AND A TECHNICAL FORMULATION: LABORATORY AND FIELD STUDIES

R. A. C. PROJECT NO. 242L

Prepared for Environment Ontario by

Environment Ontario

19/8/91



ISBN 0-7729-8270-8

ACUTE AND CHRONIC TOXICITY TO ZOOPLANKTON OF PURE PENTACHLOROPHENOL AND A TECHNICAL FORMULATION: LABORATORY AND FIELD STUDIES

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AUGUST 1991

Cette publication technique n'est disponible qu'en anglais.

PIBS 1653
log 89-9300-242

ACKNOWLEDGEMENT AND DISCLAIMER

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1.0 SUMMARY OF REPORT

There was little difference in toxicity between a technical formulation and pure pentachlorophenol to the three species of *Daphnia*; however, toxicity of both forms of PCP was influenced by duration of exposure, species and age (and/or size) of test organism, and pH of test solution. Acute toxicity of PCP was immediate, with the greatest response occurring between 0 and 24 h. The response was less between 48 and 72 h than 24 and 48 h. Pure PCP was equally toxic to all age classes of *D. magna* but susceptibility to technical PCP decreased with maturation. *D. g. mendotae* was 10 times more sensitive to PCP than *D. pulex* with *D. magna* in between having 48-h LC50 values of 1.78 and 2.57 mg/L for pure and technical PCP, respectively. Pure PCP was significantly more toxic to *D. magna* at pH 5.5 than 7.5 with mean 48-h LC50 values of 0.082 and 1.78 mg PCP/L, respectively. Control mortality of *D. magna* was higher at the lower pH and *D. magna* was intolerant of waters made basic (pH=10) by the addition of NaOH. *D. g. mendotae* could not tolerate acidic conditions (pH=5.5).

At 12 °C, the toxicity of both forms of PCP to *D. g. mendotae* and *D. pulex* did not differ significantly from that at 20 °C; however, technical PCP was significantly more toxic to *D. magna* at 12 °C for an exposure duration of 48 h. The reasons for this are unclear.

Beaker size (and/or volume of test solution) had no effect on the toxicity of PCP to *D. magna* at the lower pH of 5.5, hence adsorption to glassware was not a factor in availability of PCP to test organisms. Beaker size had no effect on the toxicity of PCP to *D. pulex* at 20 °C with test solutions having a pH of 7.0-8.0. The smaller more convenient test container of 100 mL was as consistent as the larger test containers (250-1000mL) in determining toxicity of PCP.

Chronic exposure of *D. magna* to acutely sublethal concentrations of technical and pure pentachlorophenol differentially affected maturation (i.e. time to the appearance of the primiparous instar in the brood chamber and time to the release of the first brood) and reproduction, but not survivorship or longevity. Mean number of broods produced per

daphnia, length of the reproductive period, longevity and survivorship were insensitive criteria relative to mean time to appearance of the primiparous instar in the brood chamber, time to release of the first brood, brood size and number of young produced per daphnid per reproductive day which were affected. Generally there was little difference in toxicity of the three concentrations of pure pentachlorophenol, for they significantly reduced mean brood size and the rate of production of young and significantly but differentially affected maturation. Technical PCP, at the highest concentration of 0.5 mg/L, significantly reduced mean brood size and the rate of production of young and significantly delayed both time to appearance of the primiparous instar and release of the first brood. When differences in toxicity occurred, generally, pure PCP was more toxic than comparable concentrations of technical PCP. Although enhanced maturation was observed there was no compensatory reproduction.

Similar conclusions regarding maturation, longevity and survivorship would have been derived from this study had it been terminated after 21 d; however, the conclusions would have been different for reproduction. Only the highest concentration of technical PCP reduced brood size, the rate of production of young and total number of young produced per daphnid in 21 d. Only pure PCP at 0.05 mg/L caused daphnids to produce significantly fewer broods and, although the mean brood size was significantly larger than those in the controls, the mean number of young produced in 21 d was significantly reduced. These results differ substantially from those based on the entire life cycle study and one of the most obvious differences is the much lower rates of young production in the entire life cycle study (2.41-3.03 young per daphnia per reproductive day) opposed to the 21 d study (5.13-7.5 young per daphnia per reproductive day).

The few departures in procedure from the standards detailed in the ASTM and EPA protocols for chronic toxicity testing with *Daphnia magna* were not thought to have greatly influenced the results in this study.

The half life of pentachlorophenol in waters of a mesotrophic lake (pH 8.2) was five days. Photochemical degradation was primarily responsible for the dissipation of PCP and it

was minimized by applying the chemical to the water in the evening.

PCP, at 2778 μg/L, adversely affected the phytoplankton and this was reflected by a decrease in concentrations of POC, PN, dissolved oxygen and chlorophyll *a*.

Secchi values and indices of diversity were variable and differences could not always be directly attributed to the addition of PCP.

PCP was toxic to both the macrozooplankton and the microzooplankton at 2778 μg L and the latter (rotifers) appeared to be more sensitive to PCP. PCP was likely toxic to rotifers at the nominal concentration of 1.0 mg/L. Species responded differentially to PCP and to recovery from its impact. Laboratory toxicity tests may not always accurately reflect toxicity of a chemical to the biota of an ecosystem, but they do generate results from which predictions may be based and then validated through the use of *in situ* toxicity testing methods such as limnocorrals. *In situ* toxicity testing provided, at one time, more information concerning the fate and effects of a chemical in this "pseudo-realistic" ecosystem than could be obtained from laboratory testing. The major problems, from a toxicological perspective, associated with the use of limnocorrals in this study were the variation in community structure among replicate corrals within a treatment (Experiment 1), the disparity between nominal and actual concentrations of the chemical (Experiment 2), and the failure of specific criteria to consistently reflect an impact (Secchi values, diversity and chlorophyll *a*). The lack of a consistent response was believed to be due to the complexity of and seasonal differences in interactions among components within a biological system.

2.0 ACUTE TOXICITY OF A TECHNICAL FORMULATION AND PURE PENTACHLOROPHENOL (PCP) TO THREE SPECIES OF *DAPHNIA*.

2.1 OBJECTIVES

The objectives of this study were (1) to evaluate the toxicities of a technical formulation and pure pentachlorophenol (PCP) to three age classes of *D. magna* and adult *D. g. mendotae* and *D. pulex*, and to determine the influence of exposure duration, lower temperature (12 °C) and pH (5.5), age of test organism, species of test organism and volume of test solution on the toxicity of pure and/or technical pentachlorophenol.

Daphnia magna and *Daphnia pulex* were selected as test organisms because they are easily cultured in the laboratory and therefore available throughout the year. They are cosmopolitan in distribution and important for the transfer of energy from one trophic level to another in aquatic ecosystems. They are also known to be sensitive to many toxicants, and are the test organisms recommended for use where costs must be minimized (Buikema *et al.* 1980, 1982; ASTM 1984). *Daphnia galeata mendotae* was also included because it was smaller, sensitive to pesticides (Day 1986) and present in the lake where field validation of laboratory results was to be conducted.

2.2 MATERIALS AND METHODS

2.2.1 *Preparation of pentachlorophenol*

Pure pentachlorophenol (crystalline, 99 %) was obtained from Aldrich Chemicals, Milwaukee, Wisconsin and a technical formulation (granular, 86 %) from Stanchem, Toronto, Ontario. 100-mL stock solutions with 100 g PCP/L in acetone (w:v) were contained in amber bottles, covered with aluminum foil and kept in the dark at 4 °C. These stock solutions were allowed to acclimate to room temperature and were serially diluted to 500 mL of 1000 mg

PCP/L in acetone (v:v). The second stock solutions (1000 mg/L) were placed in glass volumetric flasks, wrapped with aluminum foil, and kept in the dark at 4 °C. Prior to each experiment, these stock solutions were allowed to warm to room temperature and were used to make either 1-L or 500-mL test solutions by dilution with aerated well water from the University of Guelph (pH 7.0-7.8, alkalinity 223 ± 4.8 mg/L, particulate organic carbon 1.1 ± 0.4 mg/L and dissolved organic carbon 0.5 ± 0.1 mg/L).

2.2.2 *Acute toxicity of pure and technical PCP of three age classes of D. magna and adult D. pulex and D. g. mendotae*

Test organisms were cultured in the laboratory as described by Stephenson (1989). An acute test usually consisted of 7 to 9 treatments, 6 to 8 concentrations of PCP and controls with an acetone equivalent of the highest PCP concentration (Table 1). The daphnia used in each test were known, even-aged individuals from the breeding colony.There were five beakers per test concentration and five daphnids per beaker. Each beaker contained 80 mL of test solution and each test was repeated 5-8 times. The tests were conducted in the same environmental chamber that housed the breeding colony.

Table 1. Concentrations of pure and technical PCP (mg/L) used in the acute toxicity tests with *Daphnia*.

Species Of *Daphnia*	Test Solutions								
D. magna	Con	0.75	1.0	1.5	2.0	2.5	3.0	4.0	5.0
D. g. mendotae	Con	0.01	0.05	0.1	0.5	0.75	1.0	2.0	
D. pulex	Con	2.0	4.0	5.0	6.0	8.0	10.0		
D. magna (pH-5.5)	Con	0.001	0.006	0.01	0.06	0.1	0.6	1.0	*Con

Con represents the acetone control treatment at pH 7.0
*Con represents the acetone control treatment at pH 5.5

The assay criterion of lethality was defined as "failure to move after gently prodding with a glass rod". Observations were made after exposure durations of 24, 48 and 72 h for *D. magna* and 24 and 48 h for the other two species. Lethality was expressed as LC50 estimates derived by probit analyses of the mortality data that had been corrected for control (i.e. acetone control) mortality with Abbott's formula (James 1985; PARASTAT, PROBIT). A test was rejected if the χ^2 statistic showed the goodness-of-fit of the probit line to be inadequate (Hubert 1980). The LC50 estimates were compared using analysis of variance procedures on the log transformed data (James 1985; PARASTAT, ANOVA) and Duncan's multiple range test was used to delineate differences at $P \leq 0.05$ (James 1985; PARASTAT, DUNCAN'S N.M.R. TEST).

Concentration of dissolved oxygen, measured idiometrically (APHA 1985), and pH were determined initially before and after each test. Results showed that neither oxygen concentration nor pH differed significantly from that in the controls so these measurements were discontinued.

2.2.3 *Effects of a lower temperature on the acute toxicity of PCP to Daphnia*

A series of acute toxicity tests with adult *Daphnia* were performed at 12 ± 1 °C according to the procedures outlined above, with the following modifications. The optimum temperature for reproduction in *Daphnia* is 20 °C and reproduction decreases with decreasing temperature (Vijverberg 1980). Therefore, the individuals used in these tests were produced by the breeding colonies at 20 °C but, when they were 4-7 d old, they were transferred to the 12 °C chamber and allowed to acclimate for a period of 5-10 d. They were fed *Chlamydomonas reinhardtii, ad libitum*, and only adults were used in these tests.

2.2.4 *Effects of lower pH on the acute toxicity of pure PCP to D. magna*

Preliminary experiments were performed to define the range of toxicity of PCP in solutions with pH values of 10 and 5. *D. magna* died in water made basic by the addition of 1.0 N NaOH, but lived in water acidified by the addition of 1.0 N H_2SO_4. *D. g. mendotae* died in water acidified to pH 5.5.

Since test solutions with acidified well water tended to neutralize within hours and buffer solutions used to assess toxicity at extreme pH values were toxic to daphnia (Dave 1984), a series of experiments were conducted to determine what test solution would impart little or no mortality to the daphnids, yet maintain a relatively constant pH of 5.0–5.5. The water used in the test solutions was a mixture of distilled water and aerated well water, with a ratio of 7:3 or 5:5, respectively.

The pH of a 6-L mixture was lowered from 7.0 $\pm$ 0.5 to 5.5 $\pm$ 0.5 by the addition of 1.0 N H_2SO_4. Before the water was used to make the test solutions, it was allowed to stand for 3 h. The pH was then remeasured and, if necessary, adjusted. The concentrations of pure PCP used in these tests are listed in Table 1. There were two control treatments for each test; one control was the mixture of water at pH 7.0, before acidification, and the other control was the mixture at pH 5.5. Both control treatments had an acetone concentration equal to the highest PCP treatment. The second control was used to correct for control mortality (Abbott 1925). Other procedures used for these tests were similar to those described in section 2.2.3, except for the following modifications. In the first three experiments, the pH of the test solutions was measured every 3 h, then in subsequent tests every 12 h, and adjusted when necessary by the addition of small quantities (μL) of 0.1 N H_2SO_4 to maintain a constant pH over 48 h. Test organisms were removed from a beaker while the pH was being adjusted. No effect on survival of the daphnids could be attributed to this additional handling.

2.2.5 *Effects of beaker size and/or volume of test solution*

Not all of the acute toxicity tests with the lower pH were carried out with 80 mL of test solution in each of five replicates per treatment. Tests were also performed using 250, 600 and 1000-mL beakers with 240, 480 and 800 mL of test solution, respectively, and with 15, 30 and 50 daphnids per beaker, respectively. With these larger test containers, there were 2-4 replicates per treatment. Similar tests with beakers of different sizes were conducted at 20 °C with *D. pulex* exposed to test solutions with pH values between 7.0 and 8.0.

2.3 RESULTS

The acute toxicity of technical and pure pentachlorophenol to daphnids was influenced by duration of exposure, age of test organism and species of *Daphnia*. Young and adult *D. magna* were equally affected by technical and pure PCP (Table 2), whereas the juveniles were more susceptible to pure PCP at 48 and 72 h exposure durations (ANOVA; N=10; F=10.67 and 9.87, respectively). Technical and pure PCP were equally toxic to adult *D. pulex* and *D. g. mendotae* (Table 2). Pure PCP was equally toxic to all age classes of *D. magna*, regardless of exposure duration; but, *D. magna* young were less tolerant of technical PCP than both juveniles and adults and juveniles were significantly less tolerant than adults at 24 h (ANOVA; N=17, 17, and 12; F=13.89, 8.62 and 7.28 for 24, 48 and 72 h, respectively).

Table 2. Mean LC50 estimates (SD), in mg PCP/L, for both forms of PCP and three age classes of *D. magna* and adult *D. g. mendotae* and *D. pulex*.

Species and form of PCP	Age Class	Exposure Duration (h) 24		48		72	
D. magna							
Technical	Young	1.75	(0.33)	1.57	(0.27)	1.41	(0.34)
	Juvenile	2.55	(0.52)	2.22	(0.44)	1.83	(0.26)
	Adults	3.60	(1.05)	2.57	(0.52)	2.36	(0.32)
Pure	Young	1.87	(0.38)	1.50	(0.19)	1.37	(0.19)
	Juvenile	2.22	(0.18)	1.54	(0.15)	1.15	(0.30)
	Adult	2.63	(0.44)	1.78	(0.55)	1.27	(0.61)
D. g. m.							
Technical	Adult	0.40	(0.18)	0.33	(0.18)		
Pure		0.71	(0.16)	0.51	(0.12)		
D. pulex							
Technical	Adult	6.28	(1.04)	3.66	(0.49)		
Pure		6.83	(1.13)	4.59	(1.16)		

A comparison of LC50 estimates for adults of the three species exposed to both forms of PCP (Table 2) showed that, for each exposure duration, adult *D. g. mendotae* were significantly more sensitive than the other daphnids and that adult *D. pulex* were significantly more tolerant than *D. magna* (ANOVA; N=14-16; F=39.44-176.95).

Toxicity of pure and technical PCP to the three species of daphnia exposed at an ambient temperature of 12 °C did not, for the most part, differ significantly from those exposed at 20 °C (Figure 1). There was one exception where technical PCP was significantly more toxic to *D. magna* exposed for 48 h at 12 °C than at 20 °C, but the calculated F value of 5.58 was close to the critical value 5.32 (Figure 1).

Pure pentachlorophenol was significantly ($P \leq 0.05$) more toxic to adult *D. magna* at pH 5.5 than at 7.0 for both exposure durations of 24 h (ANOVA; N=10; F=70.25) and 48 h (ANOVA; N=10; F=60.63) (Figure 2). LC50 estimates derived for daphnids exposed to pure PCP in different volumes of test solution at pH 5.5, did not differ significantly (ANOVA; N=15; F=1.93 and 1.12 for 24 and 48 h, respectively).

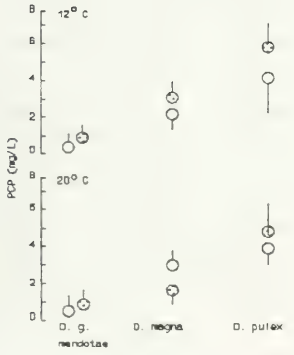

Figure 1. Mean 48-h LC50 estimates (SD) for three species of Daphnia exposed to PCP at 12 and 20 °C.

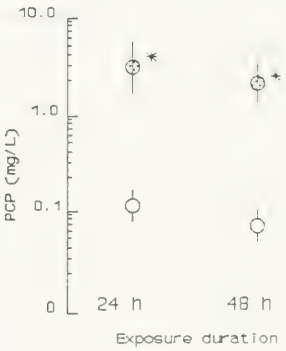

Figure 2. Mean 24- and 48-h LC50 estimates (SD) for D. magna exposed to pure PCP at pH 7.0 (O) and 5.5 (O). * Estimates differ significantly from those at the lower pH at $P \leq 0.05$.

LC50 estimates for *D. pulex* exposed to pure and technical PCP, in test solutions varying in volume, also did not differ significantly (ANOVA; N=17; F=2.63) (Figure 3).

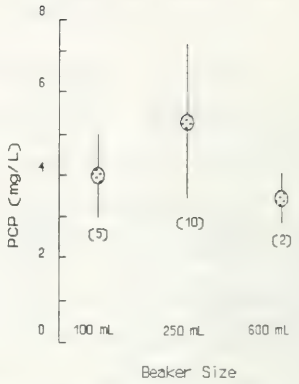

Figure 3. Mean 48-h LC50 estimates for adult D. pulex exposed to PCP in beakers of different sizes, (number of tests).

2.4 DISCUSSION

The formulation of a chemical often results in the presence of contaminants. The purity of technical formulations can vary from one company to another and even among batches produced by the same company. Buser and Bosshardt (1976) found technical formulations of PCP to be contaminated with hexa-, hepta- and octachlorodioxin, but chlorinated phenoxyphenols, diphenyl ethers and dibenzofurans may also be present (Deinzer *et al.* 1978; Nilsson *et al.* 1978; Ahlborg and Thunberg 1980; Crosby 1981). In the late 1970s and early 1980s, it was revealed that some of the symptoms observed in toxicity of technical formulations of PCP to fish were in fact the symptoms of toxicity due to the contaminants in the formulation (Nilsson *et al.* 1978; Ahlborg and Thunberg 1980; Cleveland *et al.* 1982; Huckins and Petty 1983; Johansen *et al.* 1985). In view of these results we expected that a technical formulation of PCP would be more toxic to *Daphnia* spp. than pure PCP.

Technical PCP was either as toxic to *Daphnia* or less toxic than pure PCP. This suggests that the contaminants in the technical formulation imparted no additional toxicity to the parent compound. The toxicity of both forms of PCP was influenced by exposure duration, age of test organism and species of test organism. The LC50 estimates decreased with longer exposures and, on average, the greatest change was between 24 and 48 h (mean difference 0.59 mg/L). This suggests that the initial acute response was immediate. Adema (1978) found that 48 h was the maximum time daphnids could be deprived of food before suffering

increased mortality in the presence of a toxicant (PCP), so perhaps the mortality between 48 and 72 h was compounded by the stress of food deprivation. This tends to support the contention that acute toxicity of PCP was immediate.

Neonates (≤24 h old) are generally considered to be more susceptible to toxicants than older organisms (Buikema *et al.* 1980). Assessment of the effects of age (and/or size) of test organism on the LC50 estimates of *D. magna* exposed to PCP showed an inverse relationship between age and sensitivity to PCP when there was a significant effect (Table 2). This suggests that, as daphnids mature, they may develop a capacity for tolerating technical PCP. The fact that this trend was not present in *D. magna* exposed to pure PCP (pure PCP was equally toxic to *D. magna* at all exposure durations, regardless of age), implied that there was something in the formulation that influenced this capacity for tolerance. Adema (1978) concluded from experiments with *D. magna* and pure PCP that 7-d daphnids were equally or less sensitive as day old daphnids.

Toxicity of PCP to *Daphnia* was greatly influenced by the species of test organism (Table 2). Generally, there was about a 10 fold difference in LC50 estimates between the most sensitive species, *D. g. mendotae*, and the most tolerant species *D. pulex*, with those for *D. magna* close to the median of the others. The magnitude of this variability within a single genus was similar for that among other genera (Adema and Vink 1981). Eighty percent of the freshwater invertebrate species exposed to PCP yielded LC50 values from 1 to 10 mg/L.

Toxicity of PCP is influenced by pH (Kaila and Saarikoski 1977; Kobayashi and Kishino 1980; Chapman *et al.* 1982). PCP is completely ionized at pH 9.0 (Kaiser and Valdmanis 1982). Below the pKa of 4.74 (Drakonovsky and Vacek 1971), PCP remains primarily in its undissociated or molecular form and as such is better able to penetrate biological membranes (Saarikoski and Viluksela 1981). This could account, in part, for its increased toxicity at lower pH (Spehar *et al.* 1985; Fisher and Wadleigh 1986). Most of the literature concerning the influence of pH on toxicity of PCP to aquatic organisms deals with fish (Crandall and Goodnight 1959; Kobayashi and Kishino 1980; Saarikoski and Viluksela 1981; Dave 1984; Spehar *et al.* 1985), midges (Fisher 1986; Fisher and Wadleigh 1986) and amphipods (Spehar

et al. 1985). With these tests the increased toxicity at lower pH values was consistent with that observed for *D. magna* in this study. The relative importance of this relationship will become increasingly significant as long as PCP continues to be used extensively and acidification of water systems from anthropogenic pollution continues unabated.

Fisher and Wadleigh (1986) showed that increased toxicity of PCP at lower pH values was due to differential uptake by midges and not to the presence of transformation products from increased degradation of PCP. They observed little breakdown of PCP over a 6-d period and, using radiolabelled PCP, showed that lower pH values did not enhance volatilization or adhesion to glassware. If lowering the pH of the test solution affected adsorption of the PCP to glassware, one would expect a significant effect of beaker size on the LC50 estimates. This did not occur indicating that adsorption to glassware was not a complicating factor at the lower pH of 5.5. The increased toxicity was probably due to greater penetration of biological membranes by nondissociated PCP.

Mortality, rate of residue accumulation and bioconcentration of chemicals have been correlated with changes in temperature (Cairns *et al.* 1975; Cember *et al.* 1978), primarily because of the influence of temperature on physiological processes. Frequently, higher temperatures increase metabolic requirements resulting in greater uptake of a chemical. Therefore, it was expected that the lower temperature of 12 °C would decrease filtering and feeding rates and subsequently decrease the amount of PCP being actively taken up by the daphnids. This would ultimately be expressed as higher LC50 estimates. Temperature had essentially no effect on the toxicity of both forms of PCP to the three species of *Daphnia.* with one exception (Figure 1). Technical PCP was significantly more toxic at 12 than at 20 °C for an exposure duration of 48 h which is the opposite of what we had predicted. A comparison of the log transformed slopes of the probit lines showed that the mean slope was significantly lower at 12 than 20 °C, 3.05 $\pm$ 0.61 and 5.59 $\pm$ 1.62, respectively (ANOVA; N=10; F=12.96). This difference in slopes suggested that although the LC50 was larger at the higher temperature, the range of response was very narrow. Any increase in concentration of technical PCP would result in much greater toxicity than the same increase in

concentration at 12 °C. If the criterion for toxicity had been time to lethality, then toxicity of PCP would be said to increase with increasing temperature. Brown *et al.* (1967) observed similar results for rainbow trout exposed to phenol in hard water and Crandall and Goodnight (1959) observed a decrease in mean survival time of fathead minnows exposed to sodium pentachlorophenate (Na-PCP) at higher temperatures. Chapman *et al.* (1982) found five species of aquatic oligochaetes to be less tolerant of Na-PCP at 10 °C relative to 1 and 20 °C. Fisher (1986) found pure PCP to be more toxic to midge larvae, *Chironomus riparius*, at 15 than 20 °C, but uptake of PCP was not significantly different. Nimii and Palazzo (1985) found that the rate of elimination of PCP from fish was higher at higher temperatures. The greater toxicity at the lower temperature could be caused by increased uptake, decreased rate of detoxification, decreased assimilation rates and/or a change in the susceptibility of the target site, but one would expect the same trend for pure PCP. In view of these data, we speculate that there may be something in the technical formulation that exerted a species specific influence on one or more of the previously mentioned processes, most likely the rate of detoxification. For clarification, further investigation is warranted.

3.0 CHRONIC TOXICITY OF A TECHNICAL FORMULATION AND PURE PENTACHLOROPHENOL (PCP) TO *DAPHNIA MAGNA*.

3.1 OBJECTIVES

Acute toxicity tests fail to assess the effects of prolonged exposure to lower concentrations of a chemical (Rand and Petrocelli 1985). Chronic toxicity tests with single species are the primary means of estimating potential damage of sublethal concentrations of a toxicant.

The objective of this study was to evaluate the impact of a technical formulation and pure pentachlorophenol (PCP) on maturation, reproduction and survival of *Daphnia magna* over the entire life of the organisms. This was done with the intention of comparing these results with those from a 21 d study and generating NOEC (no observable effects concentration) estimates for these two forms of PCP. Ultimately, the data could predict the chronic toxicity of PCP to zooplankton in freshwater lakes and these predictions could be subsequently validated by field studies using *in situ* enclosures.

3.2 MATERIALS AND METHODS

3.2.1 *Test design*

A static with renewal chronic toxicity test was conducted with *D. magna* exposed to four, nominal, sublethal concentrations of technical PCP (0.01, 0.05, 0.1 and 0.5 mg/L), three, nominal, sublethal concentrations of pure PCP (0.01, 0.05 and 1.0 mg/L) and a control treatment that contained acetone at a concentration equal to the highest PCP concentration. There were 20 daphnids in each of the eight treatments, one daphnid per beaker, with 80 mL of test solution in each beaker. The life cycle test was conducted at 20 ± 1 °C, with a photoperiod of 16 light : 8 dark h, from 23 January to 13 July 1986. Fluorescent cool white

lights with a CRI (Colour Rendering Index) of 68 provided a light spectrum between 290 and 700 nm and a light intensity of 65-70 $\mu E/m^2/s$ (60-65 ft candles) at the surface of the test containers.

3.2.2 *Test solutions*

One litre stock solutions of 1000 mg PCP/L, for both forms of PCP, were made by diluting the initial stock solutions of 100 g/L with pesticide grade acetone. These stock solutions were kept in the dark at 4 °C in volumetric flasks that had been wrapped with aluminum foil. The dilution water used to make the test solutions was aerated well water from the University of Guelph and was the same water in which the daphnids were cultured. The chemical composition of the dilution water was determined every second month of the study by preparing a 1-L sample according to the Analytical Methods Manual of Environment Canada (1979) and sending it to the water quality laboratory at the Canadian Centre for Inland Waters for analysis. Measurements for the various parameters are shown in Table 3. The pH of the dilution water was measured weekly with a Nester pH pen.

On 16 April 1987 the concentration of PCP was determined at 0, 24 and 48 h for 5.0-mg/L test solutions of technical and pure PCP to assess the actual concentration and the degradation of PCP under the experimental conditions. The stock solutions were also analysed to determine the actual concentrations of pentachlorophenol and 2,3,4,6-tetrachlorophenol. The analysis of these eight samples for PCP residues was performed by the Pesticide Residue Section of the Ontario Ministry of Agriculture and Food, University of Guelph, Guelph, Ontario.

Table 3. Chemical composition of the dilution water used in the chronic toxicity tests with *D. magna*.

Chemical Parameter	January	March	May	July	Mean	(SD)
Major ions (mg/L)						
Ca++	65.2	65.2	84.1	85.9	75.1	(9.9)
Cl-	28.6	28.5	27.6	27.6	28.1	(0.5)
K+	1.59	1.60	1.59	1.61	1.60	(0.01)
Na+	14.9	14.8	14.4	14.6	14.7	(0.19)
SO_4	13.8	132.0	133.9	134.1	133.2	(0.85)
Carbon (mg/L)						
DOC	0.9	0.9	0.9	0.9	0.9	(0.0)
DIC	38.8	38.5	52.1	51.8	45.2	(6.8)
POC	0.17	0.12	0.13	0.13	0.14	(0.02)
Nitrogen (μg/L)						
NO_2	*<0.2	<0.2	<0.2	<0.2	<0.2	(0.0)
NO_3	5.0	5.0	5.0	5.0	5.0	(0.0)
NH_4	127	129	95	92	110	(17.0)
TKN	78	211	65	45	99	(65.0)
PN	2	2	2	2	2	(0.0)
Phosphorus (μg/L)						
TPPF	4.9	1.7	3.0	3.2	3.2	(1.1)
TPPU	-	7.9	7.4	7.9	7.7	(0.2)
SRP	1.9	1.5	1.2	1.1	1.4	(0.3)
pH	7.8	8.2	8.1	7.7	7.9	(0.2)

* Limit of detection

Ca^{++}	calcium	NO_2	nitrite
Cl^-	chloride	NO_3	nitrate
K^+	potassium	NH_4	ammonia
Na^+	sodium	TKN	total Keldjahl nitrogen
SO_4	sulfate	PN	particulate nitrogen
DOC	dissolved organic carbon	TPPF	total particulate phosphorus filtered
DIC	dissolved inorganic carbon	TPPU	total particulate phosphorus unfiltered
POC	particulate organic carbon	SRP	soluble reactive phosphorus

3.2.3 *Test organism*

A breeding colony of *D. magna* had been housed in the environmental chamber for several generations prior to this study. A cohort of thirty 48-h old daphnids was collected and each individual placed in a 100-mL beaker with 80 mL of well water. Each daphnid was fed 3 mL of log-phase *Chlamydomonas reinhardtii* daily (Day 1986) and the water was changed every other day. The first and second broods produced were discarded, but daphnids of the third brood (24 $\pm$ 12 h old) were collected and placed in a 1-L beaker with well water and algae and left in the chamber until the next day. Therefore, the daphnids used in this study were 48 $\pm$ 12 h old.

3.2.4 *Test procedures*

The test solutions consisted of the dilution water (aerated well water), an appropriate amount of PCP, and the algae *Chlamydomonas reinhardtii* for food. Volumetric flasks (2-L), one for each test solution, were filled with approximately 1.5 L of well water. The appropriate amount of PCP was measured and transferred with volumetric pipettes from the stock solutions to these labelled flasks. Stoppers were placed on all flasks as soon as the PCP was added and each flask was shaken by inverting the flask several times. Algae, resuspended in dilution water, were then added to each flask at a rate of 2.5 mg/L and dilution water added to accurately measure 2 L. A test solution was shaken to ensure mixing and poured into each of the twenty test beakers, as well as one extra beaker which was used to minimize dilution during transfer of daphnia from the culture water to the test solutions.

The test organisms were assigned to each treatment by transferring 1-5 individuals at one time to the extra beaker in each test solution. When there were 20 daphnids per treatment, they were then randomly distributed among the test beakers, one per beaker. The beakers were placed in a transparent plastic box, one treatment per box, covered with a transparent lid and randomly placed in the environmental chamber. The test solutions

were renewed every other day with fresh test solutions in clean test beakers that were not conditioned prior to use. The daphnia were checked daily for the first 12 days, then every other day. The time to appearance of the primiparous instar in the brood chamber of the daphnia, time to release of the first brood, number of live and dead young produced, adult mortality, number of moults, appearance of ephippia and abnormal swimming behaviour were recorded.

The test beakers were cleaned thoroughly after renewal by first soaking them for 2-12 h in water with a detergent, followed by a tap water rinse, acetone rinse and/or acid bath (10 % HCl), and three rinses with deionized water with intermittent 10-20 minute baths. The acid bath removed mineral deposits.

The temperature within the environmental chamber was continually monitored with a Tempscribe, Bacharach Instrument Company, Pittsburgh, PA. and a max-min thermometer was also placed in the chamber and checked daily. The density of the algae was monitored weekly by counting the cells with a hemacytometer using the methods of Stein (1973).

3.2.5 *Statistical analyses*

Criteria used to assess chronic toxicity were mean time to appearance of the primiparous instar in the brood chamber, mean number of days to release of the first brood, mean number of broods produced per female, mean brood size per female, mean number of reproductive days, mean number of young produced per reproductive day per female and survivorship.

Univariate procedures were used to test the assumption of normality of the data for each criterion and homogeneity of variances was evaluated by plotting the calculated residuals versus the predicted values (SAS 1985; PROC UNIVARIATE, PROC PLOT). The most frequent problem associated with the data for each criterion was kurtosis. Where transformation of the data was required a log or square root transformation was performed and the data reanalyzed for normality and homeoscedasticity. Univariate procedures were also used to identify outliers and the data were reanalyzed with these values deleted. If upon

reanalysis, the outcome of a test was changed this information was presented.

A general linear models procedure, which is equal to analysis of variance with unbalanced data, with the same assumptions of independence, normality and homogeneity of variances, was used to determine significant differences at $P \leq 0.05$ among treatments for the reproduction criteria and longevity (SAS 1985; PROC GLM). Duncan's Multiple Range Test was used to delineate differences among treatment means (SAS 1985; MEANS / DUNCAN).

The survivorship data were analyzed non-parametrically with the Gehan-Wilcoxin chi-square test for significant differences between slopes of survivorship regressed against time (SAS 1985; SURVTEST).

3.3 RESULTS

3.3.1 *Concentration of PCP in stock and test solutions*

The concentration of PCP in the stock solutions used to make the test solutions of pure and technical PCP were close to the desired nominal concentration of 1000 mg/L (Table 10). PCP in the test solutions did not appear to degrade substantially over the renewal period of 48 h and the solutions of technical PCP had more 2,3,4,6-tetrachlorophenol than did the pure solutions (Table 4).

Table 4. Concentration of PCP and 2,3,4,6-tetrachlorophenol in the stock solutions of pure and technical PCP and test solutions with a nominal concentration of 5 mg PCP/L at 0, 24 and 48 h.

		Desired Nominal Concentration (mg/L)	Measured Concentration (mg/L) PCP	2,3,4,6-tetra chlorophenol
Stock solutions:				
Pure PCP		1000	1000	16
Technical PCP		1000	910	72
Test solutions:	(h)			
Pure PCP	0	5.0	4.7	0.076
	24	5.0	4.5	0.053
	48	5.0	4.7	0.067
Technical PCP	0	5.0	4.9	0.33
	24	5.0	5.2	0.42
	48	5.0	5.3	0.40

3.3.2 *Effects of PCP on reproduction of D. magna*

The mean number of broods produced per daphnia did not differ significantly among treatments (d.f.=7; F=0.74; P=0.641) (Table 5). However, the number of young produced per brood or mean brood size was significantly lower for *D. magna* exposed to 0.01 and 0.5 mg/L of technical PCP and for *D. magna* exposed to all three concentrations of pure PCP (N=158; d.f.=7; F=16.77; $P \leq 0.05$). On average, the largest broods were produced by the daphnids in the control treatment and the smallest broods by those exposed to the highest concentration of technical PCP. Brood size was significantly lower for *D. magna* exposed to pure PCP than technical PCP at 0.05 and 0.1 mg/L but not at 0.01 mg/L (Table 5). Forty-four percent of the variation in brood size could be attributed to the treatment (r^2=0.439). The residuals were normally distributed and coefficients of variation did not vary significantly among treatments (SAS, UNIVARIATE; P=0.1583).

There was no effect of technical PCP at 0.05 and 0.1 mg/L on time to appearance of the primiparous instar in the brood chamber (Table 12). At the highest concentrations of both technical and pure PCP, 0.5 and 0.1 mg/L, respectively, appearance of the primiparous instar was delayed; whereas, at the other concentrations of 0.01 mg technical PCP/L and 0.05 and 0.1 mg pure PCP/L, the time was significantly less (N=160; d.f.=7; F=18.68; $P \leq 0.05$) (Table 5). Forty-six percent of the variation in this criterion was attributable to treatment (r^2=0.463) and the coefficient of variation was 13.81 %.

Time to release of the first brood followed a similar pattern in that the highest concentrations (0.5 mg technical PCP/L, 0.05 and 0.1 mg pure PCP/L) of both forms of PCP delayed release of the first brood. Technical PCP at 0.01 mg/L did not affect time to release of the first brood; whereas, in all of the other treatments, time to release of the first brood was significantly shorter (N=160; d.f.=7; F=16.68) (Table 5). Forty four percent of the variation was attributable to treatment (r^2=0.444) and the coefficient of variation was 3.45 %.

Reproduction duration, that is the number of days for production of young, was

determined by subtracting the time to release of first brood from the number of days to death. There were no significant differences among treatment means for reproduction duration (N=160; d.f.=7; F=0.308; P=0.9494) (Table 6). However, when reproduction was considered in the context of longevity, the mean number of young produced per reproductive day per daphnid was unaffected by technical PCP at 0.01, 0.05 and 0.1 mg/L, but all three concentrations of pure PCP and 5.0 mg/L of technical PCP significantly reduced the rate of production of young (N=158; d.f.=7; F=11.13; P<0.05) (Table 5). Thirty five percent of the variation in this criterion was attributable to treatment (r^2=0.345) and the coefficient of variation was 9.48 %.

Table 5. Summary of the effects of various concentrations of both technical and pure pentachlorophenol on reproduction of *D. magna*. Values are means with SD in parentheses.

Treatments	Conc'n Of PCP (mg/L)	N	Mean Brood Size	Mean No. Of Broods	Mean No. Of Days To Release Of First Brood	Mean No. Of Days To First Appearance Of The 1° Instar	Mean No. Of Young Produced /Female/ Reproduct. Day
Controls	0	19	11.4^{a} (0.92)	24.9^{a} (8.3)	11.2^{a} (1.3)	7.4^{a} (0.9)	2.94^{ab} (0.23)
Technical PCP	0.01	20	10.6^{b} (0.74)	27.5^{a} (7.2)	10.8^{ab} (1.8)	6.7^{b} (1.0)	2.76^{bc} (0.25)
	0.05	20	11.4^{a} (1.41)	27.4^{a} (8.7)	10.2^{bc} (0.7)	7.0^{ab} (1.0)	3.03^{a} (0.32)
	0.1	19	11.2^{a} (0.81)	29.4^{a} (7.2)	10.2^{bc} (0.6)	6.8^{ab} (1.0)	2.91^{ab} (0.23)
	0.5	20	9.2^{c} (0.61)	26.2^{a} (6.7)	12.0^{d} (0.4)	8.5^{c} (1.3)	2.41^{d} (0.25)
Pure PCP	0.01	20	10.0^{b} (0.51)	27.9^{a} (6.6)	10.0^{c} (0.5)	6.5^{b} (0.9)	2.58^{c} (0.38)
	0.05	20	10.4^{b} (0.83)	25.3^{a} (7.8)	11.8^{d} (0.6)	6.5^{b} (0.9)	2.72^{c} (0.34)
	0.1	20	10.3^{b} (0.69)	27.1^{a} (6.8)	12.0^{d} (0.5)	9.1^{c} (1.0)	2.66^{c} (0.32)

Means followed by the same superscript within a column are not significantly different at P>0.05.

Table 6. Impact of various concentrations of technical and pure pentachlorophenol on longevity and the mean length of reproduction of *D. magna*. Values are means with SD in parentheses.

Treatments	Concentration Of PCP (mg/L)	N	Mean Number Of Days To Death	Mean Number Of Reproductive Days
Control	0	19	103.1 (40.0)	97 (32)
Technical PCP	0.01	20	116.8 (29.2)	106 (29)
	0.05	20	114.7 (36.0)	105 (36)
	0.1	19	116.9 (38.5)	107 (39)
	0.5	20	105.6 (35.8)	100 (27)
Pure PCP	0.01	20	111.4 (33.0)	106 (26)
	0.05	20	109.8 (34.1)	98 (34)
	0.1	20	118.0 (29.7)	106 (30)

There were no significant differences among treatment means at $P \leq 0.05$.

3.3.3 *Effects of PCP on survivorship of D. magna*

There was no significant effect of either form of PCP on average longevity (N=160; d.f.=7; F=0.51; P=0.8255) (Table 6) or on survivorship (N=160; d.f.=7; χ^2=4.93; P=0.6679) (Figure 4).

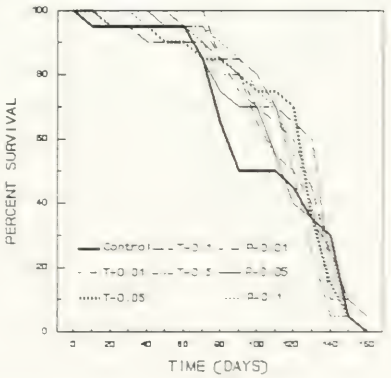

Figure 4. Mean survival of D. magna exposed to various concentrations (mg/L) of technical (T-) and pure (P-) and acetone controls for their entire lifespan.

3.4 DISCUSSION

Chronic exposure of *D. magna* to acutely sublethal concentrations of technical and pure pentachlorophenol differentially affected maturation (i.e. time to the appearance of the primiparous instar in the brood chamber and time to the release of the first brood) and reproduction, but not survivorship or longevity. Mean number of broods produced per daphnia, length of the reproductive period, longevity and survivorship were insensitive criteria relative to mean time to appearance of the primiparous instar in the brood chamber, time to release of the first brood, brood size and number of young produced per daphnid per reproductive day which were affected. Generally there was little difference in toxicity of the three concentrations of pure pentachlorophenol, for they significantly reduced mean brood size and the rate of production of young and significantly but differentially affected maturation. Technical PCP, at the highest concentration of 0.5 mg/L, significantly reduced mean brood size and the rate of production of young and significantly delayed both time to appearance of the primiparous instar and release of the first brood. When differences in toxicity occurred, generally, pure PCP was more toxic than comparable concentrations of technical PCP. Although enhanced maturation was observed there was no compensatory reproduction.

Similar conclusions regarding maturation, longevity and survivorship would have been derived from this study had it been terminated after 21 d; however, the conclusions would have been different for reproduction. Only the highest concentration of technical PCP reduced brood size, the rate of production of young and total number of young produced per daphnid in 21 d. Only pure PCP at 0.05 mg/L caused daphnids to produce significantly fewer broods and, although the mean brood size was significantly larger than those in the controls, the mean number of young produced in 21 d was significantly reduced. These results differ substantially from those based on the entire life cycle study and one of the most obvious differences is the much lower rates of young production in the entire life cycle study (2.41-3.03 young per daphnia per reproductive day) opposed to the 21 d study (5.13-7.5

young per daphnia per reproductive day).

The few departures in procedure from the standards detailed in the ASTM and EPA protocols for chronic toxicity testing with *Daphnia magna* were not thought to have greatly influenced the results in this study. The details of these departures and their implications are discussed by Stephenson (1989).

4.0 THE TOXICITY OF PENTACHLOROPHENOL (PCP) TO ZOOPLANKTON COMMUNITIES WITHIN LARGE VOLUME *IN SITU* ENCLOSURES: FIELD VALIDATION OF TOXICITY DATA DERIVED FROM LABORATORY STUDIES.

4.1 INTRODUCTION

A number of inherent limitations are frequently associated with single species toxicity test procedures (Cairns 1983). These limitations include the inability to accurately extrapolate toxicity based on a single test species to other species, the failure to demonstrate or document recovery of populations from an initial impact of a toxicant, the failure to elucidate effects of a toxicant on interactions of species within communities and probably, most importantly, the failure to accurately predict environmental impact.

In recent years, there have been attempts to develop acceptable techniques for evaluating the impact of toxicants on natural assemblages of organisms under more realistic conditions than can be simulated in the laboratory (for review see Lundgren 1985). The limnocorral technique described in detail by Solomon *et al.* (1980) and Kaushik *et al.* (1986), provides an acceptable method for assessing the impact of a toxicant on an aquatic ecosystem. Therefore, the objectives of this study were to apply PCP to limnocorrals, *in situ*, to evaluate the impact of PCP on the enclosed zooplankton, and to compare effects observed under field conditions with predicted effects based on laboratory results.

4.2 MATERIALS AND METHODS

4.2.1 *Experimental Design*

Two experiments were conducted during the ice-free months of 1987. Each consisted of six limnocorrals (5x5x4.5 m deep) that were lined with ultraviolet protected polyethylene (6 mil) and located in Lake St. George (10.3-ha, 43 57'25"N, 79 26' 30"W). The study site has been described previously (Day 1986) and details on the design, construction and installation of the limnocorrals have been described by Solomon *et al.* (1980) and Kaushik *et al.* (1986). Essentially, a limnocorral consisted of four walls, the lower ends of which were buried in the lake sediment thus effectively isolating a column of water from the surface of the lake to and including the bottom sediments.

Experiment 1 began on 28 May 1987 when the sides of the limnocorrals were lowered and sunk into the bottom sediments. On 17 June 1987 (a clear sunny day), pure pentachlorophenol (99.9 %, Aldrich Chemical, Milwaukee) dissolved in 1 L of 1 M NaOH and mixed with approximately 5 L of lake water in a garden sprayer, was applied to surface waters, between 1100 and 1300 h, to give a nominal concentration of 1 mg PCP/L. PCP was applied to three limnocorrals in a randomized block design and the other three control corrals remained untreated. This experiment ended on 4 August 1987.

A second experiment began on 6 Aug and ended on 31 October 1987. Unlike the first experiment, all fish were removed from the enclosures with a net (2 mm mesh) on 10 August. PCP was similarly applied to three enclosures between 1900 and 2100 h on 13 Aug (evening application) to achieve a nominal concentration of 2 mg PCP/L. The other three corrals served as controls.

4.2.2 *Collection of water samples for determination of chemistry and PCP residues*

Enclosures were sampled weekly during the pretreatment period and on days 1, 4, 8, 15, 22, 29 and 43 posttreatment for Experiment 1 and 1, 4, 7, 14, 21, 28, and 42 posttreatment for Experiment 2. Five 1-L water samples were collected with a vertically integrating (25-mm OD) tube sampler (Solomon *et al.* 1982) and pooled . A 1-L subsample was processed in the laboratory within 12 h and sent to the Canadian Centre for Inland Waters, Burlington, Ontario for chemical analyses.

A 1-L water subsample, from the pooled water samples collected with the integrating tube sampler (5 L), was preserved immediately in an amber bottle with approximately 1 mL of concentrated HCl and these samples were extracted and derivatized in the laboratory within 24 h.

4.2.3 *Physical and Chemical Measurements*

Temperature and oxygen were recorded in the middle of each corral with a YSI dissolved oxygen-temperature meter (model 57) at the surface and depths of 0.5, 1.5, 2.5 and 3.5 m during Experiment 1 and at the surface and 0.5, 1.0, 2.0 and 3.0 m during Experiment 2. The relative transparency of the enclosed water was ascertained by Secchi readings and pH of the water measured.

4.2.4 *Collection of zooplankton*

Five zooplankton samples were collected, in a V-shaped pattern (Stephenson *et al.* 1986) from a corral, with a vertically integrating tube sampler (150-mm OD) (Solomon *et al.* 1982), pooled, narcotized with CO_2 and preserved in the field in a 4 % sucrose-formalin solution (Haney and Hall 1973). The zooplankton were identified and counted in the laboratory as described by Stephenson *et al.* (1984), however, with the following

modifications. The macrozooplankton were counted with a compound microscope and rectangular counting chamber with a grid. Aliquots that ranged from 1 to 4 mL, depending on the density of organisms, were transferred to the counting chamber and the total subsample counted. If necessary, additional aliquots were counted up to three subsamples or 300 organisms, whichever came first.

4.2.5 *PCP Residue Analyses*

An aliquot (1 or 10 mL) of the acidified field sample, depending on the sampling date, was added to 100 mL of 0.1 M potassium carbonate solution. Twenty mL of hexane were added, followed by 1.5 mL double distilled acetic anhydride. This solution was then placed on an orbital shaker (200 rpm) for 1 h. The hexane layer was collected after filtering through sodium sulfate, to ensure removal of all water, and approximately 4 mL of isooctane added. Sample volume was reduced to approximately 1-2 mL using an analytical evaporator (nitrogen gas) and the remaining isooctane-chemical complex transferred to a volumetric flask and isooctane added to a final volume of 10 mL. An aliquot of this sample was then analyzed on a Perkin-Elmer sigma b gas chromatograph, equipped with an AS-100b autosampler and an electron capture detector. The carrier gas was hydrogen and the flow gas argon/methane. The capillary column used was a DP-5 (30 m long, fused silica, 0.25 μ film thickness). The run time was 55 min and the temperature of the column was held at 90 °C for two minutes and heated to 190 °C at a rate of 3.5 °C/min. All residue data were corrected for recovery of the PCP-acetate ester (correction factor 0.8604).

4.2.6 *Statistical Analyses*

Zooplankton were divided into two groups, the microzooplankton or Rotifera and the macrozooplankton or crustacean zooplankton which included the Cladocera, the adult Copepoda (Cyclopoids and Calanoids) and the immature Copepoda, the nauplii and

copepodites. Criteria used to evaluate the impact of pentachlorophenol on the enclosed zooplankton were numerical density, diversity of taxa and community structure via the relative percent abundances of individual species or groups of related taxa. The densities (number of organisms per unit volume) of the macro- and microzooplankton as well as the groups of taxonomically related crustacean zooplankton in the PCP-treated limnocorrals were compared with those in the control corrals by graphing mean densities, with one standard error, of replicate treatments over time. The treatment means were evaluated statistically using a nonparametric Mann-Whitney U-test comparison of ranks (Elliott 1983) or a parametric t-test on the transformed (log+1) data.

Shannon-Weaver diversity indices were calculated for the two major groups of zooplankton in each corral on each sampling date using the formula:

$$H' = - \sum_{i=1}^{s} p_i \log p_i$$

where s is the number of taxa and p_i is the proportion of total number of individuals consisting of the i^{th} taxa (Poole 1974). The numbers of taxa in each corral on each sampling date were also used as a measure of diversity.

A linear regression of the log-transformed PCP residue data followed by analysis of covariance to test for significant differences between slopes of the regression lines was used to compare the dissipation of PCP between experiments (James 1985; PARASTAT).

4.3 RESULTS

4.3.1 EXPERIMENT 1

4.3.1.1 *PCP Residues*

Analyses of water on the first day after the diurnal application of PCP indicated that the concentration of the pesticide was close to the desired nominal concentration of 1000 μg PCP/L (Figure 5).

The initial concentrations for the three corrals varied slightly, probably as a result of differences in the actual volume of water within each of the enclosures. PCP in the water had a half life of five days and by the end of the experiment (d 43) less than 1 % of the initial concentration remained. Within a couple of hours after application of PCP, the water within the treated corrals became brown in colour. This was attributed to the products of the photochemical degradation of PCP (J. Carey, pers. comm.). Dissipation of PCP from the water appeared to follow first order kinetics.

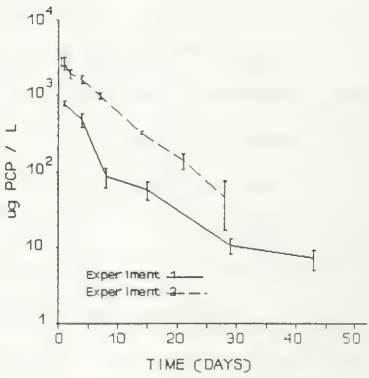

Figure 5. Mean (±SE) concentration of pentachlorophenol in water of enclosures in Experiments 1 and 2.

4.3.1.2 *Physical and chemical parameters*

There was no change in pH of the water exposed to pentachlorophenol (Table 6a) and the temperature profiles at two depths were similar in all corrals for both experiments (Table 6a).

Table 6a. Ranges of the values for pH and temperature at 0.5 and 3.5 or 3.0 m for control and PCP-treated corrals for Experiments 1 and 2.

Parameter	Treatment	Experiment 1	Experiment 2
pH	Controls	7.87-8.37	8.1-8.2
	PCP	7.83-8.30	8.0-8.1
Temperature at 0.5 m	Controls	20.0-26.0	17.3-25.3
	PCP	20.0-25.3	17.0-25.0
Temperature at 3 or 3.5	Controls	15.2-19.5	17.0-23.7
	PCP	14.8-19.2	18.2-23.5

Secchi readings varied greatly among replicate corrals for both the controls and the PCP-treated corrals; however, greatest divergence between treatments occurred immediately after the pesticide was applied when there was greater transparency of water in the PCP-treated corrals (Figure 6).

Mean concentration of oxygen in the water at a depth of 0.5 m showed a decline in the PCP-treated corrals immediately posttreatment (Figure 6), but again the variability among the replicate enclosures was extremely high, especially for the control corrals so this difference was not statistically significant ($P>0.05$). At the lower depth of 3.5 m, there seemed to be little difference in oxygen concentration and the tremendous variability associated with the mean values was attributed to the presence of a hypolimnion in two of the enclosures (Figure 6).

Chlorophyll *a* concentration was also highly variable within replicate enclosures during the pretreatment period (Figure 6). Although there was less variation during the posttreatment period, there was no discernable impact of PCP on mean chlorophyll levels.

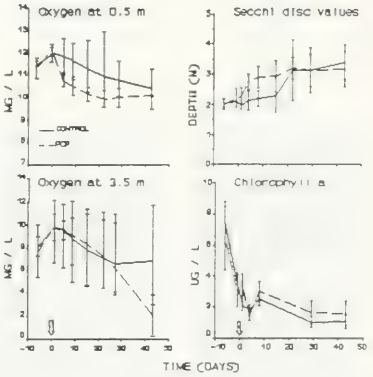

Figure 6. Mean (±SE) concentration of DO, Secchi readings and concentration of chlorophyll a in control and PCP-treated corrals for Experiment 1, ⇩ application of PCP.

The chemical parameters of the water also appeared to be, for the most part, unaffected by the addition of PCP (Table 7), with some notable exceptions. The concentration of chlorine ions was significantly higher for the entire posttreatment period in the corrals treated with PCP. Carbon (POC) and nitrogen (PN) of particulate matter decreased immediately after PCP was applied but this decrease was short lived with recovery to levels similar to those in the controls by d 20.

Table 7. Mean and range for the chemical parameters of the water in the control and PCP-treated enclosures in Experiment 1.

Major ions (mg/L)	Controls Mean	Controls Range	PCP Mean	PCP Range
Ca++	59.8	68.3-42.7	61.4	68.3-48.3
Cl-	12.3	13.4-10.7	12.7	14.4-10.8
K+	1.9	1.9- 1.8	1.9	2.0- 1.8
Na+	5.5	6.3- 4.8	5.6	6.4- 4.7
SO_4	16.4	17.9-15.0	16.5	18.2-15.0
Carbon (mg/L)				
POC	1.8	2.4- 1.2	1.5	2.4- 1.0
DIC	37.9	43.4-26.5	39.3	43.9-31.6
DOC	7.3	11.5- 6.3	7.0	7.8- 6.6
Nitrogen (µg/L)				
NO_2	1.3	2.6- 0.7	1.8	2.9- 1.4
NO_3	13.0	20.4- 9.1	13.7	20.5- 9.5
NH_4	30.0	85.0- 6.0	33.0	85.0-13.0
PN	165.0	219.0-100.0	146.0	221.0-70.0
TKN	486.0	733.0-194.0	579.0	685.0-42.0
Phosphorus (µg/L)				
TPPF	10.5	22.1- 5.8	10.5	16.1- 8.4
TPPU	21.9	30.6-13.1	24.8	33.9- 9.3
SRP	1.0	1.7- 0.3	1.2	1.9- 0.8
Chlorophyll (µg/L)	3.0	7.5- 1.0	3.1	6.5- 1.5

Ca^{++}	calcium	NO_2	nitrite
Cl^-	chloride	NO_3	nitrate
K^+	potassium	NH_4	ammonia
Na^+	sodium	TKN	total Keldjahl nitrogen
SO_4	sulfate	PN	particulate nitrogen
DOC	dissolved organic carbon	TPPF	total particulate phosphorus filtered
DIC	dissolved inorganic carbon	TPPU	total particulate phosphorus unfiltered
POC	particulate organic carbon	SRP	soluble reactive phosphorus

4.3.1.3 *Zooplankton density and diversity*

PCP at 1.0 mg/L was not acutely toxic to the macrozooplankton and the mean numerical densities in the treated enclosures did not differ significantly from those in the control corrals (Figure 7). However, there appeared to be a decline in densities of the microzooplankton immediately posttreatment followed by recovery to levels similar to those in the control corrals within 15 d (Figure 7). This observed difference was not statistically significant (t-test, n=12, P>0.05) given the high degree of variation associated with the population estimates. The average coefficient of variation (C.V.) associated with the rotifer density estimates throughout Experiment 1 was 57 and 80 % (n=18) for the control and PCP-treated corrals, respectively. Similar trends were observed for the three major groups that comprised the macrozooplankton.

The relative percent abundances of the four major groups of zooplankton in control and PCP-treated corrals reflected the nature of the dissimilarity among replicates and may explain some of the observed variability in density estimates (Figure 8). Control corrals 2 and 12 had communities

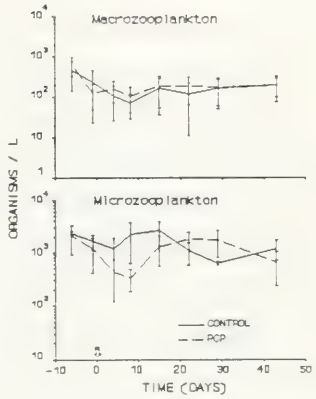

Figure 7. Changes in mean densities (±SE) of zooplankton in control and PCP-treated enclosures for Experiment 1, ⇓ application of PCP.

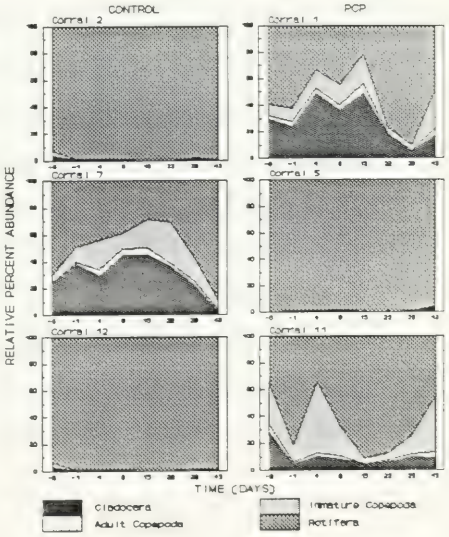

Figure 8. Relative percent abundances of the four major groups of zooplankton in replicate control and PCP-treated corrals during Experiment 1.

that were numerically dominated by rotifers, whereas corral 7 was equally represented by crustacean zooplankton and rotifers. The three corrals treated with PCP had entirely different communities with rotifers comprising almost the entire zooplankton community in corral 5. Numbers in corral 1 were, for the most part, split evenly between the macro- and microzooplankton, whereas corral 11 had a much greater proportion of immature Copepoda.

This dissimilarity among replicate enclosures within both treatments was also evident at the species level. *Bosmina longirostris* was the most prevalent macrozooplankton species in the three control corrals during the pretreatment period. However on d 4, corral 7 began to deviate from the others as *Ceriodaphnia lacustris* and *Daphnia galeata mendotae* became more prevalent. By d 8 *C. lacustris* was the most abundant macrozooplankton taxon in this corral and remained so throughout the experiment. A concurrent deviation of the microzooplankton community also occurred in corral 7 with this community being represented by a greater number of taxa, but no one taxon became consistently dominant from one week to another. *Keratella cochlearis*. *Polyarthra spp.* and toward the end of the experiment (d 22-43) *Conochiloides sp.* were the most abundant rotifer taxa present in the control corrals. *K. cochlearis* and *Polyarthra spp.* also dominated the rotifer communities in the corrals to which PCP was added. Toward the end of the experiment (d 22-43) *Conochilus unicornis* was present with a conspicuous absence of *Conochiloides sp.*. *B. longirostris* was frequently the most abundant macrozooplankton species present in the three treated corrals; however, on days 22 to 43 corral 11 contained greater abundances of *D. g. mendotae* and *Diaptomus oregonesis*.

Diversity of taxa within the two groups of zooplankton, as indicated by the Shannon-Weaver index and the number of taxa present in a sample did not reveal a significant impact of PCP on these criteria. These data were extremely variable (Stephenson 1989).

4.3.2 EXPERIMENT 2

4.3.2.1 *PCP residues*

Analyses of water on the first day following the evening application of the pesticide indicated that the concentration was higher than the desired nominal concentration of 2000 μg PCP/L (Figure 5). The amount of pesticide added was calculated with the assumption that the volume of water in an enclosure was 100 m^3. If the volume of water was less than this, then the concentration of PCP would inevitably have been higher. The mean depth in the centre of the three treated corrals in Experiment 1 was 4.5 m, whereas in Experiment 2 it was 4 m. The lake level gradually decreased over the summer and the corrals were not as deep during the second experiment.

The half life of PCP in water following the evening application was also five days and dissipation of PCP did not differ from that in Experiment 1 since there was no significant difference between slopes of the regression lines (ANOCOVA; F=57.05; d.f.=1,38). However, the mean concentration (SD) of PCP in the treated enclosures at 8 h was 2778 (294) μg L and by 18 h it had only decreased to 2681 (418) μg/L which suggests that little photolytic degradation of PCP had occurred within the first 24 h following the evening application of PCP. The same brown colour that appeared in Experiment 1 following the diurnal application of PCP gradually appeared in the surface waters of the enclosures as the sun rose and by late afternoon on d 1 the waters in the treated enclosures were brown. The rate of dissipation of PCP from the water was similar in both experiments after the first 24 h.

4.3.2.2 *Physical and chemical parameters*

As in Experiment 1, mean pH and temperature values for water to which PCP was added did not differ from those in the control corrals (Table 6).

Secchi values indicated a gradual increase in water transparency over the experimental

period in the control enclosures. There was a slight increase in water transparency following the evening application of PCP, but on d 20, waters in the treated enclosures were significantly ($P \leq 0.05$) less clear than those in the control enclosures.

A decline in the concentration of dissolved oxygen in water at 0.5 and 3.5 m initially appeared to be caused by PCP; however, a similar trend was observed in the control corrals about one week later. Although there was a posttreatment decline on d 4, there was no statistically significant impact of PCP on concentrations of chlorophyll. As in the first experiment, few water chemistry parameters were affected by the addition of PCP (Table 8). Both POC and PN declined immediately posttreatment with significant differences occurring on days 4 and 7 for POC ($P \leq 0.05$). However, as in Experiment 1, the decline was extremely short in duration and concentrations began to increase after d 4 and exceeded levels in the controls which were gradually declining after the first week of the experiment.

4.3.2.3 *Zooplankton density and diversity*

Following the evening application of PCP there was a significant decrease in density of both the macrozooplankton and the microzooplankton (Figure 9), and all groups within the macrozooplankton were similarily affected. Recovery of populations to levels similar to those in the controls occurred on d 20 and d 28 for the microzooplankton and macrozooplankton, respectively.

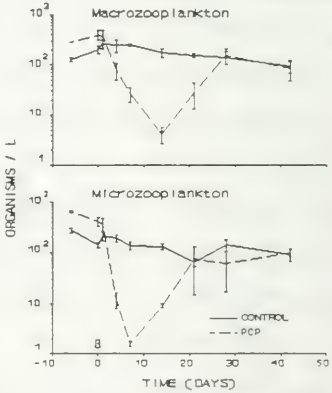

Figure 9. Changes in mean densities (±SE) of zooplankton in control and PCP-treated corrls for Experiment 2, ⇓ application of PCP.

Table 8. Mean and range for the chemical parameters of the water in the control enclosures and those treated with 2.0 mg PCP/L.

Major ions (mg/L)	Controls Mean	Controls Range	PCP Mean	PCP Range
Ca++	52.2	58.1-47.4	53.15	5.3-48.3
Cl-	12.5	13.5-10.8	13.8	15.8-10.8
K+	1.8	1.9- 1.8	1.9	2.0- 1.8
Na+	5.7	6.4- 4.7	6.1	6.9- 4.7
SO_4	16.1	17.6-13.5	16.1	17.7-13.6
Carbon (mg/L)				
DOC	1.3	2.0- 0.5	1.2	2.3- 0.5
DIC	33.8	37.6-28.1	33.9	36.7-29.1
POC	7.3	7.7- 6.9	7.5	8.0- 6.9
Nitrogen (μg/L)				
NO_2	1.3	3.0- 0.7	2.1	4.7- 0.5
NH_4	47.0	119.0- 5.0	94.3	799.0- 7.0
PN	118.0	172.0-84.0	150.3	283.0-74.0
TKN	678.0	1583.0-539.0	689.0	1034.0-511.0
Phosphorus (μg/L)				
TPPF	11.2	38.9- 6.7	17.3	78.2- 7.6
TPPU	22.5	41.2-14.0	27.3	59.5- 9.4
SRP	1.3	7.3- 0.4	2.1	16.8- 0.7
Chlorophyll (μg/L)	2.1	3.7- 0.4	2.3	9.5- 0.1

Ca^{++}	calcium	NO_2	nitrite
Cl^-	chloride	NO_3	nitrate
K^+	potassium	NH_4	ammonia
Na^+	sodium	TKN	total Keldjahl nitrogen
SO_4	sulfate	PN	particulate nitrogen
DOC	dissolved organic carbon	TPPF	total particulate phosphorus filtered
DIC	dissolved inorganic carbon	TPPU	total particulate phosphorus unfiltered
POC	particulate organic carbon	SRP	soluble reactive phosphorus

The variability associated with the density estimates of zooplankton in this experiment was less than that in Experiment 1, with mean coefficients of variation of 23 and 45 % (n=36) for controls and PCP-treated enclosures, respectively. The relative percent abundances of the four major groups of zooplankton reflected a greater similarity in community structure among the three control corrals (Figure 11). The three PCP-treated enclosures initially appeared to be similar with respect to community structure, however they became increasingly dissimilar toward the end of the experiment. Corral 5 became markedly different with the total absence of immature Copepoda from d 21-42.

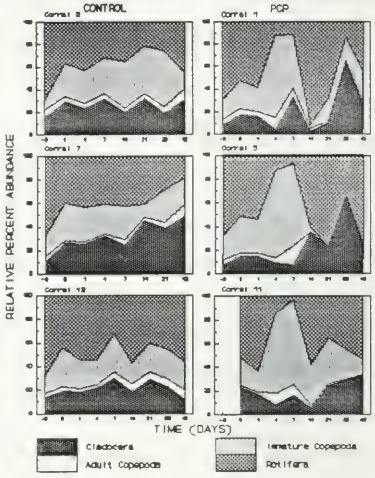

Figure 10. Relative percent abundance of the four major groups of zooplankton in replicate control and PCP-treated corrals for Experiment 2.

The species composition of the macrozooplankton communities was also similar for all three control corrals and the communities were numerically dominated by the Cladocera. *D. g. mendotae*, *C. lacustris*, *B. longirostris* and *Diaphanosoma brachyurum* were most prevalent in the first part of the experiment with a gradual increasing dominance of *D. g. mendotae*. *Diaptomus oregonensis* became more abundant between d 28 and 42 than previously. The increase in relative percent abundance of *D. oregonensis* in the control corrals did not occur in the PCP-treated corrals despite this taxa being present in the first part of the experiment. *Tropocyclops p. mexicanus* became relatively more abundant immediately posttreatment in the enclosures treated with PCP, then it became conspicuously less abundant from d 14-42. The microzooplankton communities within the PCP treatment were similar for about one week after treatment, however they deviated upon initiation of recovery.

Kellicottia bostoniensis comprised 50 % of the rotifer population on d 14 in corral 5 and one

week later, on d 21, *Cephalodella sp.* made up 50 %. On d 28 the same enclosure was dominated by *Synchaeta sp.* The weekly changes in composition of the rotifer taxa during this recovery phase appeared to be variable and unpredictable which was not the case for the recovery of the macrozooplankton. By d 21, all three PCP-treated corrals had macrozooplankton communities numerically dominated by *D. g. mendotae* and all three corrals had very dissimilar microzooplankton populations with *Polyarthra spp., K. cochlearis,* and *Cephalodella sp.* being proportionally prevalent in corral 11 and *Cephalodella sp.* and *Polyarthra spp.* numerically dominant in corrals 5 and 1, respectively. By d 42 Polyarthra spp. was common to all three enclosures but in different proportions.

Diversity of taxa within the macrozooplankton was not affected significantly by PCP; however, diversity within the microzooplankton, as measured by the Shannon-Weaver index, declined from the beginning of the experiment in the treated corrals and, with one exception (d 8), remained lower than the controls for the duration of the experiment. Significant differences in diversity of taxa between the control and PCP-treated corrals (Mann-Whitney U test, n=24, $P \leq 0.05$) occurred on days 0, 1, 4 and 43.

4.4 DISCUSSION

4.4.1 DISSIPATION OF PCP FROM WATER

In light of the evidence presented by Crossland and Wolff (1985) on the chemical nature and behaviour of PCP in aquatic environments, we expected to see a rapid dissipation of PCP in Experiment 1 and predicted a half life of between 2-4 days.There was some agreement between expected and observed effects. The 5 d half life of PCP in water was close to the predicted halflife. The brown colour that appeared in the water within 2 h suggested that PCP was photochemically degraded while it was more concentrated near the surface. In Experiment 2, we expected photolysis of the PCP applied to the surface in the evening to be minimized. The chemical would have had time to penetrate deeper into the water column

prior to the onset of photochemical degradation. Since rate of dissipation of PCP is independent of concentration, the rate would not change from that of Experiment 1 (i.e. a half-life of 2-4 d), but the initial concentration of PCP encountered by zooplankton would be highest during the first 12 h when sunlight was absent. The small change (3.5 %) in concentration of PCP, between the pre-dawn, 8-h posttreatment sample and the 18-h posttreatment sample (10 h of exposure to sunlight), suggested that photolysis had been minimized. A concurrent study of the photodegradation of technical tetrachlorophenol (TTCP) by Liber *et al.* (unpublished) indicated that there was greater penetration of the water column by the chemical prior to the onset of photodegradation. Comparable data were not available from the first experiment but if one assumed the maximum concentration to be 1000 μg/L, then the measured concentration of PCP in water at 24 h represents a reduction of 21.7 %. Dissipation of PCP appeared to follow first order kinetics and was very similar to that described by Crossland and Wolff (1985).

4.4.2 TOXICITY OF PCP TO BIOTA

The acute toxicity of pure pentachlorophenol to three age classes of *D. magna* and adult *D. g. mendotae* and *D. pulex* was determined using standardized, static, 48-h toxicity tests (Section 2.0, Table 4). Pure pentachlorophenol was toxic to adult *D. magna*, *D. g. mendotae* and *D. pulex* at 1.78, 0.51 and 4.59 mg/L (48h-LC50), respectively. It was equally toxic to young (24-48 h) and juvenile (3-4 d) *D. magna* with 48h-LC50 estimates of 1.50 and 1.54 mg PCP/L, respectively. The 48h-LC50 estimates for the three species of daphnia, which were present in Lake St. George, provided an expected response range on which to base predictions regarding toxicity to zooplankton of 1.0 mg PCP/L. On the basis of our laboratory results, we predicted differential toxicity to the Cladocera with some species exhibiting less resilience than others. A subsequent decline in diversity of taxa was also expected. On the basis of past experiments with pesticides applied to limnocorrals in this lentic system (Kaushik *et al.* 1985; Stephenson *et al.* 1986; Day 1986), we predicted there

would be little impact of PCP on rotifer densities because rotifers, as a group, appear to be more tolerant of pesticides (Hurlbert *et al.* 1972; Miura and Takahashi 1976; Papst and Boyer 1980).

The observed effects in Experiment 1 did not validate these predictions. The lack of toxicity to the cladoceran crustacean was as unexpected as was the apparent sensitivity of the rotifers. We were expecting approximately 50 % mortality of the macrozooplankton and observed essentially no mortality. Nevertheless, these observations can be explained, in part, by the mode and time of application of PCP. Since the laboratory results clearly indicated that the lethal response of daphnids to PCP occurred over an extremely narrow range of concentrations, it was suspected that the rapid photodegradation of PCP in Experiment 1 quickly drove the concentration below the acute NOEC of 700 μg PCP/L. Schauerte *et al.* (1982) observed complete mortality of daphnia within 3 d of applying nominal concentrations of 1 and 5 mg PCP/L to ponds. The initial exposure concentration of 783 μg PCP/L in this study was close to that measured by Schauerte *et al.* (1982), but they did not report the pH of the water in their "ponds" so it is difficult to speculate on the nature of this apparent disparity. PCP was not toxic to Daphniidae at chronic concentrations ranging from 5 to 139 μg/L (Crossland and Wolff 1985) and there was no observed toxicity to Copepoda exposed to concentrations of 7, 76 and 622 μg PCP/L (Cantelmo and Rao 1978).

There was a large amount of variation associated with the mean estimates of plankton densities in Experiment 1 (Figures 7) which obscured what probably was a significant impact of PCP on rotifers. This variation was the result of differences in community structure within replicate corrals (Figure 8). Planktivorous fish, which were inadvertently enclosed and unevenly distributed among corrals, were believed to be responsible for the reduction of macrozooplankton and subsequent domination of corrals 2, 12 and 5 by the Rotifera (Figure 8). The removal of fish from the enclosures in the second experiment was expected to reduce the variation in community structure among corral replicates. Smaller coefficients of variation associated with the density estimates as well as relatively greater similarity in community structure within the control corrals (Figures 9 and 10) attested to this.

In Experiment 2, 60-80 % mortality of the macrozooplankton was expected from exposure to the nominal concentration of 2000 μg PCP/L. The problem with rapid photolysis of the chemical had been minimized by applying the PCP in the evening so that the actual exposure concentration was expected to be close to the nominal concentration. A decline in rotifer densities was expected since results from Experiment 1 suggested that they were sensitive to PCP. The problem encountered in this experiment was that the volume of water in two of the three PCP enclosures was less than expected, hence the measured concentrations of PCP exceeded the desired nominal concentration (Figure 5). This emphasizes the importance of concurrently measuring the actual exposure concentrations of the chemical during field studies.

PCP at 2778 μg/L was acutely toxic to the microzooplankton and macrozooplankton which began to recover by d 7 and 14, respectively, when the mean concentration of PCP in water was 982 and 322 μg/L, respectively. There had been no attempt to predict recovery of plankton populations from the impact of PCP, however, these data suggest that recovery may commence when concentrations of the chemical are close to or below the NOEC determined by acute toxicity tests in the laboratory. Although time to intiation of recovery for the three groups of macrozooplankton was d 14, the Cladocera recovered at a faster rate relative to adult and immature Copepoda, primarily because of their shorter generation time.

Species responded differentially to PCP. The increased prevalence of *T. p. mexicanus* immediately posttreatment suggested that this cyclopoid crustacean was more tolerant of the chemical. On the other hand, failure of *D. oregonensis* to become more prevalent in the PCP-treated corrals toward the end of the experiment reflected the inability of this univoltine species to "recover" quickly from toxicity. The population dynamics observed for rotifers during the recovery phase of Experiment 2, also reflected individual differences among species with regard to reproductive potential and capacity to recover.

Toxicity of pentachlorophenol to phytoplankton and periphyton has been demonstrated (Knowlton and Huckins 1983; Crossland and Wolff 1985; Yount and Richter 1986). The concentrations of PCP used in this study exceeded most of the LC50 estimates

derived from laboratory toxicity tests with algae. Therefore, we expected a decrease in concentration of dissolved oxygen as evidence that photosynthesis had been affected by PCP, a decrease in chlorophyll *a* resulting from direct toxicity to peri- and phytoplankton and an increase in Secchi readings as waters in the enclosures cleared due to phytotoxicity and subsequent sedimentation. A decrease in concentration of dissolved oxygen at 0.5 m, an increase in clarity of water and no impact on chlorophyll *a* levels were observed in Experiment 1, but again differences could not be statistically validated because of the extreme variability within treatment replicates. In Experiment 2, dissolved oxygen concentrations at both depths appeared to reflect an impact of PCP on photosynthesis. The concentration of chlorophyll *a* briefly declined immediately posttreatment which suggested that there was direct toxicity to phytoplankton at 2778 μg PCP/L but that populations recovered rather quickly. There was no corresponding increase in clarity of water.

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